

Cardiovascular Effects of Arsenic:
Clinical and Epidemiological Findings

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Abstract

Several population studies relate exposure to high levels of arsenic with an increased incidence of ischemic heart disease and cardiovascular mortality. An association has been shown between exposure to high levels of arsenic and cardiovascular risk factors such as hypertension and diabetes mellitus, and vascular damage such as subclinical carotid atherosclerosis. The mechanisms underlying these phenomena are currently being studied and appear to indicate an alteration of vascular function. However, the effects of low levels of exposure to arsenic and their potential detrimental cardiovascular effect are less explored. The article provides an overview of the pathophysiologic mechanisms linking low-level arsenic exposure to the occurrence of cardiovascular disease and its complications, and some potential preventive strategies to implement.

Keywords

Arsenic, Cardiovascular disease, Environmental pollution, Drinking water, Oxidative stress, Toxicology

Arsenic (As) is a natural element of the earth's crust whose toxicity has been known for centuries. Adverse effects depend on dose, duration and frequency of exposure, and can range from neurological and respiratory damage to the onset of cancers of the skin, lung and bladder (Brunton LL 2011; Longo DL 2012): the International Agency for Research on Cancer has classified inorganic As as a group 1 human carcinogen (WHO-IARC 2012). Occupational exposure and intentional poisoning are well-known sources of acute toxicity, whereas lower-level long-standing exposures require a broad range of expertise for As detection and the study of environmental fate and mechanisms of human toxicity. The main cause of chronic intoxication for the general population is ingestion of drinking water drilled in deposits contaminated with this metalloid, occurring mainly in developing countries, but also to a lesser extent in the Western world (Lisabeth et al. 2010; Medrano et al. 2010). The World Health Organization and most national legislations have set a limit of 10 µg/L of As in drinking water, corresponding to the reliable detection threshold using current methods, while some countries (especially developing countries) still maintain a threshold of 50 µg/L as recommended by WHO before 1993.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) set a Provisional Tolerable Intake (PTWI) for inorganic As at 2.1 µg/Kg/day or 15 µg/Kg/week (JECFA 1989). The European Food Safety Authority Panel on Contaminants in the Food Chain (EFSA-CONTAM) noted that after this PTWI was established, new data showed that inorganic As causes cancer of the lung and urinary tract in addition to skin, and a range of adverse effects have been reported at exposures lower than those reviewed by the JECFA, concluding that the PTWI of 15 µg/Kg was not appropriate. In 2010 the JECFA withdrew the PTWI (JECFA 2011). The main adverse effects reported by the EFSA-CONTAM Panel associated with long-term ingestion of inorganic As in humans were skin lesions, cancer, developmental toxicity, neurotoxicity, cardiovascular (CV) disease, abnormal glucose metabolism and diabetes. On the other hand, evidence of CV disease and diabetes in areas with relatively low levels of inorganic As exposure was evaluated as inconclusive (EFSA-CONTAM 2009).

High levels of As exposure in drinking water have been related to increased risk of CV disease. Since this is a major cause of death worldwide, and most of the world's population resides in developing countries where the incidence of CV disease is rising, even a modest increase in risk due to As in groundwater might have substantial impact on morbidity and mortality.

The fate of As in the human body

The metalloid As is a natural environmental contaminant. It is present in organic and inorganic form,

and in different valence or oxidation states – mainly the trivalent (III) and pentavalent (V). The most toxicologically potent As compounds are in the trivalent oxidation state. Organic arsenicals in the pentavalent oxidation state – such as those used as pesticides, even today – are much less toxic than inorganic ones, because unlike inorganic As they are not readily taken up by cells, and undergo limited metabolism (Cohen et al. 2006; Hughes et al. 2011); concern regarding these compounds is due to their progressive breakdown to inorganic As in the environment. As is ubiquitous; diet is the main source of total As for humans, but the organic compounds normally present in food, especially in fish and seafood, are considered non-toxic or of low toxicity. Populations living in coastal areas have on average higher urinary excretion of As than those living inland, without this in itself turning out in toxicity (Delgado-Andrade et al. 2003; EFSA-CONTAM 2009; JECFA 2011; WHO-IARC 2012). Total urinary As is an unreliable marker of exposure to inorganic As, the most toxic form, so studies often adjust this measurement for seafood consumption (Navas-Acien et al. 2008; Kim and Lee 2011; Moon et al. 2012). Soil, air, and absorption through the skin are a minor source of As for the general population, but may be important in professional exposure.

Conversely, As found in water is almost entirely inorganic, and drinking water accounts for most exposure to this form. In water As is not in its elemental form, which is insoluble, but as arsenate (pentavalent) in surface water, while arsenite (trivalent) is more prevalent in deep anoxic wells (Flora 2011; WHO-IARC 2012). Metabolization in the human body is not completely understood, but the main step is methylation, primarily in the liver, with or without the involvement of glutathione (GSH) (Watanabe and Hirano 2012); most excretion in the urine is in the form of dimethyl, a smaller part as methyl, while about a quarter is excreted as inorganic (Vahter 2000). While methylation is a detoxification process (Huang et al. 2009), some of the intermediate metabolites, particularly those with trivalent As, could mediate some of the toxic and carcinogenic effects of As.

The precise mode of action of As toxicity is even less well-known. Several modes of action, possibly interrelated, have been proposed: interaction with sulfur and binding to sulfhydryl groups on proteins and enzymes; substitution of arsenate for phosphate, since As and phosphorus both belong to group 15 of the periodic table and share physiochemical properties, cutting off ATP formation in particular; substitution for zinc in zinc fingers; genotoxic action, potentiating the mutagen action of other compounds but also inhibiting DNA enzymatic repair and altering DNA methylation; and alteration of cellular signal transduction (Hughes et al. 2011; Flora 2011). The formation of reactive oxygen species (ROS), also occurring at concentrations that are not directly cytotoxic (Barchowsky et al. 1996), could be the pathogenic mechanism for the observed CV effects.

As and CV disease

Several epidemiological studies in different countries have found a relationship between exposure to high levels of As – as high as $> 50 \mu\text{g/L}$ in drinking water, but the limits used to define “high”, “moderate” or “low” referring to non-professional exposure vary widely – and CV disease. The relationship is particularly evident for peripheral arterial disease (“blackfoot disease” is a form of PAD endemic to areas of Taiwan with extremely high levels of As in drinking water (Tseng 1977)) and coronary heart disease, while the association with stroke is less strong and of borderline significance (Lisabeth et al. 2010; Moon et al. 2012). The studies globally show a temporal sequence between time of exposure and onset of disease, have yielded consistent results in different countries and populations, show a dose-response relation, and show decreased incidence of disease where reduced exposure has been implemented (Chang et al. 2004; Yuan et al. 2007). The association between As and CV disease is at least partly independent of traditional risk factors; prospective studies do confirm the association (Chen et al. 2011b) and various biological mechanisms for CV toxicity have been investigated; all this is enough to define a causal relationship, i.e., a high level of As exposure is an established risk factor for CV disease (Sapoznik 2010; Moon et al. 2012).

Less is known about the CV effects of exposure to lower As levels, closer to the WHO threshold, which interests a much larger population worldwide. Results are conflicting; at these levels, the magnitude of the effect is expected to be smaller, and methodological limitations – mainly the difficulty of assessing actual and cumulative individual exposure, but also the distinction between organic and inorganic As, and outcome measures with the potential confounders – may have played a role in the non-significance of most statistical associations performed so far.

As in the CV continuum

As has been associated in a dose-dependent manner with high blood pressure (Wang et al. 2011; Abhyankar et al. 2012), even at low levels of exposure (Zhang et al. 2012a), and with renal dysfunction (Chen et al. 2011a) and proteinuria (Chen et al. 2011c). The association with diabetes has been limited to sufficient evidence for high levels (Maull et al. 2012), while some recent data (Jovanovic et al. 2012) conducted with more precise exposure measurements assessing the relation with glucose metabolism (Del Razo et al. 2011) suggest an association even at lower levels (Huang et al. 2011; Maull et al. 2012). The pathophysiological mechanism could be a direct toxic effect on beta-cells, interaction with cellular signaling, or enzyme inhibition in beta-cells or in peripheral tissues (Huang et al. 2011). Consistent with

the association with risk factors and CV disease, an association has also been found between As exposure and atherosclerosis (Wang et al. 2002; Wang et al. 2010; Hsieh et al. 2011), and confirmed in experimental animal studies (Bunderson et al. 2004; Srivastava et al. 2009; Lemaire et al. 2011).

Endothelial dysfunction: the link between As and CV disease?

Oxidative stress is an established and widely studied mechanism of As toxicity. ROS are formed both in vivo and in vitro in the presence of As. They include superoxide anion, hydroxyl radical, hydrogen peroxide, reactive nitrogen species, and As-centered and As peroxy radicals (Bao and Shi 2010b; Hughes et al. 2011; Flora 2011). The mechanisms of ROS formation by As are not completely clear: they may be produced during oxidation of arsenite (III) to arsenate (V) during metabolism, by stimulation of the enzymes NADH or NADPH oxidase (Smith et al. 2001), and NOX (States et al. 2009), or by mobilization of free iron from ferritin (Shi et al. 2004; Flora 2011; Jomova et al. 2011). Nitric oxide (NO) is one of the main mediators released from the endothelium. It has vasodilatory and anti-inflammatory properties, and inhibits platelet adhesion and aggregation, as well as smooth muscle cell proliferation and migration. ROS inactivate NO and reduce its bioavailability in the vascular endothelium. In addition to the direct action of ROS on NO, As inhibits endothelial NO synthetasis (eNOS) (Fig. 1) (Lee et al. 2003; Tsou et al. 2005; Li et al. 2007), it depletes GSH – one of the main antioxidant ROS scavengers – and inhibits redox enzymes (catalase, glutathione peroxidase and reductase, thioredoxin reductase, superoxide dismutase). The disruption of antioxidant defense mechanisms and the blocking of new NO formation further decrease NO bioavailability and increase endothelial dysfunction (Pi et al. 2000). It is notable that acute exposure or low doses do actually induce redox enzymes, indirectly confirming their role in As detoxification (Shi et al. 2004; Flora 2011). Consistently with the oxidative stress frame, several antioxidants have shown, in vivo or in vitro, a protective effect against As-induced toxicity. These include N-acetylcysteine (Bao and Shi 2010a), alpha-lipoic acid, vitamin C and E, taurine, quercetin (Ghosh et al. 2011), some plant extracts including garlic (Balakumar and Kaur 2009; Biswas et al. 2010; Flora 2011), and resveratrol (Zhao et al. 2008b; Zhang et al. 2012b).

When NO concentrations are reduced, the endothelial surface becomes more prone to atherosclerosis, cell adhesion and thrombosis, a condition known as endothelial dysfunction, common to all major CV risk factors (aging, hypertension, diabetes, dyslipidemia, etc.) (Deanfield et al. 2007). One of the mechanisms by which As induces endothelial dysfunction is by exaggerating calcium influx in cells (Suriyo et al. 2012). Exposure to As augments endothelial permeability through increased VEGF

expression (Bao and Shi 2010a) and reduces proteoglycan synthesis in endothelial cells (Fujiwara et al. 2005); individuals drinking water contaminated with As show dose-dependent expression of plasma levels of cell adhesion molecules (CAM), i.e., a pro-atherosclerotic endothelium (Chen et al. 2007). While it is known that As enhances oxidative stress, and that oxidative stress produces endothelial dysfunction, the direct link between exposure to As and endothelial dysfunction in humans has not been investigated extensively. There are no published studies on As and in vivo endothelial function, either in small vessels (venous plethysmography) (Joannides et al. 2006) or conduit vessels (flow-mediated dilation) (Deanfield et al. 2007).

Other mechanisms of CV damage

Endothelial dysfunction characterizes arterial hypertension, and pharmacological blockade of eNOS by L-NMMA raises blood pressure (Owlya et al. 1997), so diminished NO availability is a plausible mechanism to link As with hypertension (Hsueh et al. 2005). Moreover, As decreases in vitro vascular relaxation capability, both endothelium-dependent and endothelium-independent, directly acting on the smooth muscle cells (Lee et al. 2003), while it potentiates response to vasoconstrictors through myosin light chain phosphorylation and Ca^{++} sensitization/inflow (Lee et al. 2005; Li et al. 2010; Lim et al. 2011). Interaction with arachidonic acid biotransformation, influencing vasoconstriction and relaxation, may be another mechanism (Bunderson et al. 2004). Renal damage may be involved in raising blood pressure (Chen et al. 2011c; Hsueh et al. 2009), but the causal relationship with this association remains to be clarified.

As exposure in a wide range of levels is consistently associated with QT prolongation – a risk factor for arrhythmia and sudden cardiac death – torsade de pointes (Ficker et al. 2004; Mumford et al. 2007; Mordukhovich et al. 2009; Wang et al. 2009), and increased QT dispersion (Wang et al. 2010). As may act on QT by increasing cardiac calcium currents (Ficker et al. 2004), but no protective effect of calcium-antagonists has been found (Mordukhovich et al. 2009) and there are conflicting data on verapamil enhancing or reducing As cardiotoxicity (Zhao et al. 2008a; Luong and Rabkin 2009). Through the abovementioned mechanisms, As also exerts its direct cytotoxic effects on cardiomyocytes, inducing apoptosis or necrosis (Zhao et al. 2008a; Luong and Rabkin 2009); moreover, the myocardium appears to be a sensitive target tissue for As (Roman et al. 2011). However, population data are scarce, while cardiotoxicity of As_2O_3 as a chemotherapeutic drug is well-known, and is more marked in Afro-Americans, probably due to genetic susceptibility (Patel et al. 2006). Specific effects of As on the heart could explain the disproportionate increase in CV events compared to stroke despite the known effect on

blood pressure (Zierold et al. 2004; Lisabeth et al. 2010). Although there appear to be no studies regarding a relationship between heart failure or cardiomyopathy and As, there are hints of a synergistic toxic effect of As and alcohol on the heart (Navas-Acien et al. 2006; Bao and Shi 2010b).

Polymorphisms in the genes of enzymes involved in As metabolism, especially As methyltransferase, and in redox enzymes can modulate As toxicity and its CV effects (Vahter 2000; Hsueh et al. 2005; States et al. 2009; Hsieh et al. 2011; Hughes et al. 2011)

Reduced synthesis of extracellular matrix and damage to vascular smooth muscle cells are other mechanisms that could promote atherosclerotic plaque formation and instability (Hays et al. 2008; Li et al. 2010), while oxidation of lipids would make them more prone to be internalized in the vessel wall (Srivastava et al. 2009). As appears to enhance platelet aggregation (Lee et al. 2002), possibly by exposing phosphatidylserine (Bae et al. 2009) though studies are conflicting (Lin et al. 2010), and reduces endothelial fibrinolytic activity by reducing t-PA and increasing PAI-1 level and activity (Jiang et al. 2002), and reduces synthesis of proteoglycans such as heparan sulfate (Fujiwara et al. 2005). The activity of As on coagulation and platelets could aggravate thrombosis when the atherosclerotic plaque ruptures (Fig. 2).

CV toxicity of As: diagnosis and therapy

CV toxicity due to As exposure can be suspected based on personal history. Professional exposure is a concern for people working in mines, the wood industry, coal-fired power plants, ore smelting, battery assembly, and electronics. Intentional poisoning was considered a “perfect crime” in the past since As is odorless and tasteless and symptoms of acute intoxication are quite aspecific, but its use has become rare since As is now detectable in fluids and tissues (Hughes et al. 2011). Contamination of drinking water is usually well-known in particular areas. The patient should be carefully questioned and examined for other As-associated diseases, such as skin lesions and cancers. Hints from clinical history and examination must be confirmed by evidence of As levels in body fluids and tissues indicative of exposure (Das and Sengupta 2008; Marchiset-Ferlay et al. 2012). Since As is metabolized from the blood within a period of hours, blood As levels – normally < 1 µg/l – are not a good indicator of long-term exposure, and they are only partially correlated with As in drinking water. The majority of absorbed As is excreted in the urine, normally within 1-2 days, so that urinary As is considered a reliable marker of recent exposure (Jomova et al. 2011). However, as previously mentioned not all forms of As are (equally) toxic, so total urinary As should be considered only a screening test, or suitable for population studies or continuous professional exposure. Distinguishing organic from

inorganic forms, and between oxidation states, requires more complex methods such as chromatographic techniques or spectrometry, and special care in managing the sample – adjusting for seafood consumption, the major source of organic As, is neither reliable nor plausible on a routine basis at the individual level. Studying As in toenails and hair, where trivalent As binds with sulfhydryl groups in keratin, provides information on a more long-term exposure. As species are more stable in these samples, and most of the As is inorganic; on the other hand, the correlation between toenail As and exposure to inorganic As is poor (Hinwood et al. 2003; Slotnick and Nriagu 2006). Variable growth rate of skin appendages could be another confounding factor (Das and Sengupta 2008; Marchiset-Ferlay et al. 2012).

There are no guidelines or consensus statements that tell the clinical cardiologist when to screen for As exposure, but aside from confirmation of exposure suspected on the basis of history or environment, a reasonable approach might be to search for As in patients when arrhythmias or CV events occur without obvious cause or in absence of traditional risk factors.

Patients diagnosed with As poisoning should be promptly removed from sources of the metalloid and provided with supportive measures and specific care for the diseases developed, along with treatment of symptoms and correction of nutritional deficits (States et al. 2009) and electrolyte imbalance, which increases the risk for arrhythmias. The most effective specific treatment for arsenicosis is chelation therapy (Brunton LL 2011; Longo DL 2012); however, it is limited to life-threatening conditions due to its potentially severe side effects. Most of the effects of antioxidants have been tested either in vitro, on animals, or (in humans) on soft endpoints (Biswas et al. 2010) but there is some indication of their usefulness as adjunctive therapy during chelation, especially for N-acetylcysteine (Flora et al. 2007).

Conclusions

Several studies suggest CV effects of As exposure at low-to-moderate levels. These effects may translate into a higher CV mortality; however, studies on mortality from CV disease have been inconsistent. Although the mechanisms through which As can cause atherosclerotic lesions and their complications have been explored, individual risks from environmental pollution remain to be established. Many factors make the assessment of such a relationship quite difficult, such as the means of measurement, confounding factors from conventional risk factors for CV disease, and the variability of threshold allowed by individual countries for As pollution. It is time to move from population to individual risk assessment in order to profile the real impact of As exposure at low doses through imaging biomarkers of atherosclerosis. More prospective studies designed to assess doses (thresholds)

and risks and individual susceptibility through genetic testing are needed to explore the relationship between As exposure and the occurrence of CV disease and its complications.

Legend to figures

Fig. 1. Arsenic and endothelial dysfunction

ATG: angiotensinogen, AT: angiotensin, ACE: angiotensin converting enzyme, ET: endothelin, COX: cyclooxygenase, PG: prostaglandin, TX: tromboxane, NADPH: nicotinamide dinucleotide phosphate, eNOS: endothelial nitric oxide synthase, NO: nitric oxide, cGMP: cyclic guanosine monophosphate, EDHF: endothelium-derived hyperpolarizing factors

Fig. 2. Arsenic and mechanisms of CV damage

SMC: smooth muscle cells, GSH: glutathione, t-PA: tissue plasminogen activator, PAI-1: plasminogen activator inhibitor type 1

Bibliography

- Abhyankar LN, Jones MR, Guallar E, Navas-Acien A (2012) Arsenic exposure and hypertension: a systematic review. *Environ Health Perspect* 120 (4):494-500. doi:10.1289/ehp.1103988
- Bae ON, Lim KM, Noh JY, Chung SM, Kim SH, Chung JH (2009) Trivalent methylated arsenical-induced phosphatidylserine exposure and apoptosis in platelets may lead to increased thrombus formation. *Toxicol Appl Pharmacol* 239 (2):144-153. doi:S0041-008X(08)00526-7 [pii] 10.1016/j.taap.2008.12.020
- Balakumar P, Kaur J (2009) Arsenic exposure and cardiovascular disorders: an overview. *Cardiovasc Toxicol* 9 (4):169-176. doi:10.1007/s12012-009-9050-6
- Bao L, Shi H (2010a) Arsenite induces endothelial cell permeability increase through a reactive oxygen species-vascular endothelial growth factor pathway. *Chem Res Toxicol* 23 (11):1726-1734. doi:10.1021/tx100191t
- Bao L, Shi H (2010b) Potential molecular mechanisms for combined toxicity of arsenic and alcohol. *J Inorg Biochem* 104 (12):1229-1233. doi:S0162-0134(10)00189-3 [pii] 10.1016/j.jinorgbio.2010.08.005
- Barchowsky A, Dudek EJ, Treadwell MD, Wetterhahn KE (1996) Arsenic induces oxidant stress and NF-kappa B activation in cultured aortic endothelial cells. *Free Radic Biol Med* 21 (6):783-790. doi:0891584996001748 [pii]
- Biswas J, Sinha D, Mukherjee S, Roy S, Siddiqi M, Roy M (2010) Curcumin protects DNA damage in a chronically arsenic-exposed population of West Bengal. *Hum Exp Toxicol* 29 (6):513-524. doi:0960327109359020 [pii] 10.1177/0960327109359020
- Brunton LL CB, Knollmann BC (2011) Goodman & Gilman's The Pharmacological Basis of Therapeutics. 12th edn. McGraw-Hill,
- Bunderson M, Brooks DM, Walker DL, Rosenfeld ME, Coffin JD, Beall HD (2004) Arsenic exposure exacerbates atherosclerotic plaque formation and increases nitrotyrosine and leukotriene biosynthesis. *Toxicol Appl Pharmacol* 201 (1):32-39. doi:S0041008X04002261 [pii] 10.1016/j.taap.2004.04.008
- Chang CC, Ho SC, Tsai SS, Yang CY (2004) Ischemic heart disease mortality reduction in an arseniasis-endemic area in southwestern Taiwan after a switch in the tap-water supply system. *J Toxicol Environ Health A* 67 (17):1353-1361. doi:10.1080/15287390490471451

BFCE878WJ0Q997JG [pii]

Chen JW, Chen HY, Li WF, Liou SH, Chen CJ, Wu JH, Wang SL (2011a) The association between total urinary arsenic concentration and renal dysfunction in a community-based population from central Taiwan. *Chemosphere* 84 (1):17-24. doi:S0045-6535(11)00256-6 [pii]

10.1016/j.chemosphere.2011.02.091

Chen Y, Graziano JH, Parvez F, Liu M, Slavkovich V, Kalra T, Argos M, Islam T, Ahmed A, Rakibuz-Zaman M, Hasan R, Sarwar G, Levy D, van Geen A, Ahsan H (2011b) Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study. *BMJ* 342:d2431

Chen Y, Parvez F, Liu M, Pesola GR, Gamble MV, Slavkovich V, Islam T, Ahmed A, Hasan R, Graziano JH, Ahsan H (2011c) Association between arsenic exposure from drinking water and proteinuria: results from the Health Effects of Arsenic Longitudinal Study. *Int J Epidemiol* 40 (3):828-835. doi:dyr022 [pii]

10.1093/ije/dyr022

Chen Y, Santella RM, Kibriya MG, Wang Q, Kappil M, Verret WJ, Graziano JH, Ahsan H (2007) Association between arsenic exposure from drinking water and plasma levels of soluble cell adhesion molecules. *Environ Health Perspect* 115 (10):1415-1420. doi:10.1289/ehp.10277

Cohen SM, Arnold LL, Eldan M, Lewis AS, Beck BD (2006) Methylated arsenicals: the implications of metabolism and carcinogenicity studies in rodents to human risk assessment. *Crit Rev Toxicol* 36 (2):99-133

Das NK, Sengupta SR (2008) Arsenicosis: diagnosis and treatment. *Indian J Dermatol Venereol Leprol* 74 (6):571-581

Deanfield JE, Halcox JP, Rabelink TJ (2007) Endothelial function and dysfunction: testing and clinical relevance. *Circulation* 115 (10):1285-1295. doi:115/10/1285 [pii]

10.1161/CIRCULATIONAHA.106.652859

Del Razo LM, Garcia-Vargas GG, Valenzuela OL, Castellanos EH, Sanchez-Pena LC, Currier JM, Drobna Z, Loomis D, Styblo M (2011) Exposure to arsenic in drinking water is associated with increased prevalence of diabetes: a cross-sectional study in the Zimapan and Lagunera regions in Mexico. *Environ Health* 10:73. doi:1476-069X-10-73 [pii]

10.1186/1476-069X-10-73

Delgado-Andrade C, Navarro M, Lopez H, Lopez MC (2003) Determination of total arsenic levels by hydride generation atomic absorption spectrometry in foods from south-east Spain: estimation

of daily dietary intake. *Food Addit Contam* 20 (10):923-932.
doi:10.1080/02652030310001594450
0D9URCTCR6JF53N7 [pii]

EFSA-CONTAM (2009) Scientific Opinion on Arsenic in Food *EFSA Journal* 7 (10):1351.
doi:10.2903/j.efsa.2009.1351

Ficker E, Kuryshev YA, Dennis AT, Obejero-Paz C, Wang L, Hawryluk P, Wible BA, Brown AM
(2004) Mechanisms of arsenic-induced prolongation of cardiac repolarization. *Mol Pharmacol*
66 (1):33-44. doi:10.1124/mol.66.1.33
66/1/33 [pii]

Flora SJ (2011) Arsenic-induced oxidative stress and its reversibility. *Free Radic Biol Med* 51 (2):257-
281. doi:S0891-5849(11)00234-6 [pii]
10.1016/j.freeradbiomed.2011.04.008

Flora SJ, Bhadauria S, Kannan GM, Singh N (2007) Arsenic induced oxidative stress and the role of
antioxidant supplementation during chelation: a review. *J Environ Biol* 28 (2 Suppl):333-347

Fujiwara Y, Nakase Y, Kaji T (2005) Sodium arsenite inhibits proteoglycan synthesis by vascular
endothelial cells in culture. *Journal of health science* 51 (4):461-468

Ghosh S, Dungdung SR, Chowdhury ST, Mandal AK, Sarkar S, Ghosh D, Das N (2011) Encapsulation
of the flavonoid quercetin with an arsenic chelator into nanocapsules enables the simultaneous
delivery of hydrophobic and hydrophilic drugs with a synergistic effect against chronic arsenic
accumulation and oxidative stress. *Free Radic Biol Med* 51 (10):1893-1902. doi:S0891-
5849(11)00532-6 [pii]
10.1016/j.freeradbiomed.2011.08.019

Hays AM, Lantz RC, Rodgers LS, Sollome JJ, Vaillancourt RR, Andrew AS, Hamilton JW, Camenisch
TD (2008) Arsenic-induced decreases in the vascular matrix. *Toxicol Pathol* 36 (6):805-817.
doi:0192623308323919 [pii]
10.1177/0192623308323919

Hinwood AL, Sim MR, Jolley D, de Klerk N, Bastone EB, Gerostamoulos J, Drummer OH (2003)
Risk factors for increased urinary inorganic arsenic concentrations from low arsenic
concentrations in drinking water. *Int J Environ Health Res* 13 (3):271-284.
doi:10.1080/0960312031000122424
M5DYWDA4BTAGVFGW [pii]

- Hsieh YC, Lien LM, Chung WT, Hsieh FI, Hsieh PF, Wu MM, Tseng HP, Chiou HY, Chen CJ (2011) Significantly increased risk of carotid atherosclerosis with arsenic exposure and polymorphisms in arsenic metabolism genes. *Environ Res* 111 (6):804-810. doi:S0013-9351(11)00120-4 [pii] 10.1016/j.envres.2011.05.003
- Hsueh YM, Chung CJ, Shiue HS, Chen JB, Chiang SS, Yang MH, Tai CW, Su CT (2009) Urinary arsenic species and CKD in a Taiwanese population: a case-control study. *Am J Kidney Dis* 54 (5):859-870. doi:S0272-6386(09)00879-8 [pii] 10.1053/j.ajkd.2009.06.016
- Hsueh YM, Lin P, Chen HW, Shiue HS, Chung CJ, Tsai CT, Huang YK, Chiou HY, Chen CJ (2005) Genetic polymorphisms of oxidative and antioxidant enzymes and arsenic-related hypertension. *J Toxicol Environ Health A* 68 (17-18):1471-1484. doi:V0184UG57U6865W1 [pii] 10.1080/15287390590967414
- Huang CF, Chen YW, Yang CY, Tsai KS, Yang RS, Liu SH (2011) Arsenic and diabetes: current perspectives. *Kaohsiung J Med Sci* 27 (9):402-410. doi:S1607-551X(11)00117-3 [pii] 10.1016/j.kjms.2011.05.008
- Huang YL, Hsueh YM, Huang YK, Yip PK, Yang MH, Chen CJ (2009) Urinary arsenic methylation capability and carotid atherosclerosis risk in subjects living in arsenicosis-hyperendemic areas in southwestern Taiwan. *Sci Total Environ* 407 (8):2608-2614. doi:S0048-9697(09)00011-4 [pii] 10.1016/j.scitotenv.2008.12.061
- Hughes MF, Beck BD, Chen Y, Lewis AS, Thomas DJ (2011) Arsenic exposure and toxicology: a historical perspective. *Toxicol Sci* 123 (2):305-332. doi:kfr184 [pii] 10.1093/toxsci/kfr184
- JECFA (1989) Evaluation of certain food additives and contaminants. Thirty-third Report of the Joint FAO/WHO Expert Committee on Food Additives. *World Health Organ Tech Rep Ser* 776:1-64
- JECFA (2011) Evaluation of certain contaminants in food. *World Health Organ Tech Rep Ser* (959):1-105, back cover
- Jiang SJ, Lin TM, Wu HL, Han HS, Shi GY (2002) Decrease of fibrinolytic activity in human endothelial cells by arsenite. *Thromb Res* 105 (1):55-62. doi:S0049384801003978 [pii]
- Joannides R, Bellien J, Thuillez C (2006) Clinical methods for the evaluation of endothelial function--a focus on resistance arteries. *Fundam Clin Pharmacol* 20 (3):311-320. doi:FCP406 [pii] 10.1111/j.1472-8206.2006.00406.x

- Jomova K, Jenisova Z, Feszterova M, Baros S, Liska J, Hudecova D, Rhodes CJ, Valko M (2011) Arsenic: toxicity, oxidative stress and human disease. *J Appl Toxicol* 31 (2):95-107. doi:10.1002/jat.1649
- Jovanovic D, Rasic-Milutinovic Z, Paunovic K, Jakovljevic B, Plavsic S, Milosevic J (2012) Low levels of arsenic in drinking water and type 2 diabetes in Middle Banat region, Serbia. *Int J Hyg Environ Health*. doi:S1438-4639(12)00002-8 [pii] 10.1016/j.ijheh.2012.01.001
- Kim Y, Lee BK (2011) Association between urinary arsenic and diabetes mellitus in the Korean general population according to KNHANES 2008. *Sci Total Environ* 409 (19):4054-4062. doi:S0048-9697(11)00624-3 [pii] 10.1016/j.scitotenv.2011.06.003
- Lee MY, Bae ON, Chung SM, Kang KT, Lee JY, Chung JH (2002) Enhancement of platelet aggregation and thrombus formation by arsenic in drinking water: a contributing factor to cardiovascular disease. *Toxicol Appl Pharmacol* 179 (2):83-88. doi:10.1006/taap.2001.9356 S0041008X01993561 [pii]
- Lee MY, Jung BI, Chung SM, Bae ON, Lee JY, Park JD, Yang JS, Lee H, Chung JH (2003) Arsenic-induced dysfunction in relaxation of blood vessels. *Environ Health Perspect* 111 (4):513-517
- Lee MY, Lee YH, Lim KM, Chung SM, Bae ON, Kim H, Lee CR, Park JD, Chung JH (2005) Inorganic arsenite potentiates vasoconstriction through calcium sensitization in vascular smooth muscle. *Environ Health Perspect* 113 (10):1330-1335
- Lemaire M, Lemarie CA, Molina MF, Schiffrin EL, Lehoux S, Mann KK (2011) Exposure to moderate arsenic concentrations increases atherosclerosis in ApoE^{-/-} mouse model. *Toxicol Sci* 122 (1):211-221. doi:kfr097 [pii] 10.1093/toxsci/kfr097
- Li B, Sun Y, Sun X, Wang Y, Li X, Kumagai Y, Sun G (2007) Monomethylarsonous acid induced cytotoxicity and endothelial nitric oxide synthase phosphorylation in endothelial cells. *Bull Environ Contam Toxicol* 78 (6):455-458. doi:10.1007/s00128-007-9178-7
- Li JX, Shen YQ, Cai BZ, Zhao J, Bai X, Lu YJ, Li XQ (2010) Arsenic trioxide induces the apoptosis in vascular smooth muscle cells via increasing intracellular calcium and ROS formation. *Mol Biol Rep* 37 (3):1569-1576. doi:10.1007/s11033-009-9561-z

- Lim KM, Shin YS, Kang S, Noh JY, Kim K, Chung SM, Yun YP, Chung JH (2011) Potentiation of vasoconstriction and pressor response by low concentration of monomethylarsonous acid (MMA(III)). *Toxicol Lett* 205 (3):250-256. doi:S0378-4274(11)01272-0 [pii]
10.1016/j.toxlet.2011.06.008
- Lin KH, Chang YF, Fan CY, Jayakumar T, Lee JJ, Chou DS, Hsiao G, Sheu JR (2010) Arsenic trioxide-mediated antiplatelet activity: pivotal role of the phospholipase C gamma 2-protein kinase C-p38 MAPK cascade. *Transl Res* 155 (2):97-108. doi:S1931-5244(09)00263-1 [pii]
10.1016/j.trsl.2009.08.005
- Lisabeth LD, Ahn HJ, Chen JJ, Sealy-Jefferson S, Burke JF, Meliker JR (2010) Arsenic in drinking water and stroke hospitalizations in Michigan. *Stroke* 41 (11):2499-2504. doi:STROKEAHA.110.585281 [pii]
10.1161/STROKEAHA.110.585281
- Longo DL FA, Kasper D, Hauser S, Jameson JL, Loscalzo J (2012) *Harrison's Principles of Internal Medicine*. 17th edn. McGraw-Hill,
- Luong MW, Rabkin SW (2009) Verapamil but not calpain or creatine alters arsenate-induced cardiac cell death. *Toxicol Ind Health* 25 (3):169-176. doi:25/3/169 [pii]
10.1177/0748233709105593
- Marchiset-Ferlay N, Savanovitch C, Sauvant-Rochat MP (2012) What is the best biomarker to assess arsenic exposure via drinking water? *Environ Int* 39 (1):150-171. doi:S0160-4120(11)00216-9 [pii]
10.1016/j.envint.2011.07.015
- Maull EA, Ahsan H, Edwards J, Longnecker MP, Navas-Acien A, Pi J, Silbergeld EK, Styblo M, Tseng CH, Thayer KA, Loomis D (2012) Evaluation of the Association between Arsenic and Diabetes: A National Toxicology Program Workshop Review. *Environ Health Perspect*. doi:10.1289/ehp.1104579
- Medrano MA, Boix R, Pastor-Barriuso R, Palau M, Damian J, Ramis R, Del Barrio JL, Navas-Acien A (2010) Arsenic in public water supplies and cardiovascular mortality in Spain. *Environ Res* 110 (5):448-454. doi:S0013-9351(09)00191-1 [pii]
10.1016/j.envres.2009.10.002
- Moon K, Guallar E, Navas-Acien A (2012) Arsenic Exposure and Cardiovascular Disease: An Updated Systematic Review. *Curr Atheroscler Rep*. doi:10.1007/s11883-012-0280-x

- Mordukhovich I, Wright RO, Amarasiriwardena C, Baja E, Baccarelli A, Suh H, Sparrow D, Vokonas P, Schwartz J (2009) Association between low-level environmental arsenic exposure and QT interval duration in a general population study. *Am J Epidemiol* 170 (6):739-746. doi:kwp191 [pii]
10.1093/aje/kwp191
- Mumford JL, Wu K, Xia Y, Kwok R, Yang Z, Foster J, Sanders WE (2007) Chronic arsenic exposure and cardiac repolarization abnormalities with QT interval prolongation in a population-based study. *Environ Health Perspect* 115 (5):690-694. doi:10.1289/ehp.9686
- Navas-Acien A, Sharrett AR, Guallar E (2006) THREE AUTHORS REPLY. *American Journal of Epidemiology* 164 (2):195-196. doi:10.1093/aje/kwj198
- Navas-Acien A, Silbergeld EK, Pastor-Barriuso R, Guallar E (2008) Arsenic exposure and prevalence of type 2 diabetes in US adults. *JAMA* 300 (7):814-822. doi:300/7/814 [pii]
10.1001/jama.300.7.814
- Owlya R, Vollenweider L, Trueb L, Sartori C, Lepori M, Nicod P, Scherrer U (1997) Cardiovascular and sympathetic effects of nitric oxide inhibition at rest and during static exercise in humans. *Circulation* 96 (11):3897-3903
- Patel SP, Garcia-Manero G, Ferrajoli A, Faderl S, Verstovek S, Kantarjian H, Estey E (2006) Cardiotoxicity in African-American patients treated with arsenic trioxide for acute promyelocytic leukemia. *Leuk Res* 30 (3):362-363. doi:S0145-2126(05)00307-3 [pii]
10.1016/j.leukres.2005.08.002
- Pi J, Kumagai Y, Sun G, Yamauchi H, Yoshida T, Iso H, Endo A, Yu L, Yuki K, Miyauchi T, Shimojo N (2000) Decreased serum concentrations of nitric oxide metabolites among Chinese in an endemic area of chronic arsenic poisoning in inner Mongolia. *Free Radic Biol Med* 28 (7):1137-1142. doi:S0891-5849(00)00209-4 [pii]
- Roman DA, Pizarro I, Rivera L, Camara C, Palacios MA, Gomez MM, Solar C (2011) An approach to the arsenic status in cardiovascular tissues of patients with coronary heart disease. *Hum Exp Toxicol* 30 (9):1150-1164. doi:0960327110389835 [pii]
10.1177/0960327110389835
- Saposnik G (2010) Drinking water and risk of stroke: the hidden element. *Stroke* 41 (11):2451-2452. doi:STROKEAHA.110.596395 [pii]
10.1161/STROKEAHA.110.596395

- Shi H, Shi X, Liu KJ (2004) Oxidative mechanism of arsenic toxicity and carcinogenesis. *Mol Cell Biochem* 255 (1-2):67-78
- Slotnick MJ, Nriagu JO (2006) Validity of human nails as a biomarker of arsenic and selenium exposure: A review. *Environ Res* 102 (1):125-139. doi:S0013-9351(05)00190-8 [pii]
10.1016/j.envres.2005.12.001
- Smith KR, Klei LR, Barchowsky A (2001) Arsenite stimulates plasma membrane NADPH oxidase in vascular endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 280 (3):L442-449
- Srivastava S, Vladykovskaya EN, Haberzettl P, Sithu SD, D'Souza SE, States JC (2009) Arsenic exacerbates atherosclerotic lesion formation and inflammation in ApoE^{-/-} mice. *Toxicol Appl Pharmacol* 241 (1):90-100. doi:S0041-008X(09)00333-0 [pii]
10.1016/j.taap.2009.08.004
- States JC, Srivastava S, Chen Y, Barchowsky A (2009) Arsenic and cardiovascular disease. *Toxicol Sci* 107 (2):312-323. doi:kfn236 [pii]
10.1093/toxsci/kfn236
- Suriyo T, Watcharasit P, Thiantanawat A, Satayavivad J (2012) Arsenite promotes apoptosis and dysfunction in microvascular endothelial cells via an alteration of intracellular calcium homeostasis. *Toxicol In Vitro* 26 (3):386-395. doi:S0887-2333(11)00343-2 [pii]
10.1016/j.tiv.2011.12.017
- Tseng WP (1977) Effects and dose--response relationships of skin cancer and blackfoot disease with arsenic. *Environ Health Perspect* 19:109-119
- Tsou TC, Tsai FY, Hsieh YW, Li LA, Yeh SC, Chang LW (2005) Arsenite induces endothelial cytotoxicity by down-regulation of vascular endothelial nitric oxide synthase. *Toxicol Appl Pharmacol* 208 (3):277-284. doi:S0041-008X(05)00113-4 [pii]
10.1016/j.taap.2005.03.001
- Vahter M (2000) Genetic polymorphism in the biotransformation of inorganic arsenic and its role in toxicity. *Toxicol Lett* 112-113:209-217. doi:S0378427499002714 [pii]
- Wang CH, Chen CL, Hsiao CK, Chiang FT, Hsu LI, Chiou HY, Hsueh YM, Wu MM, Chen CJ (2009) Increased risk of QT prolongation associated with atherosclerotic diseases in arseniasis-endemic area in southwestern coast of Taiwan. *Toxicol Appl Pharmacol* 239 (3):320-324. doi:S0041-008X(09)00264-6 [pii]
10.1016/j.taap.2009.06.017

- Wang CH, Chen CL, Hsiao CK, Chiang FT, Hsu LI, Chiou HY, Hsueh YM, Wu MM, Chen CJ (2010) Arsenic-induced QT dispersion is associated with atherosclerotic diseases and predicts long-term cardiovascular mortality in subjects with previous exposure to arsenic: A 17-Year follow-up study. *Cardiovasc Toxicol* 10 (1):17-26. doi:10.1007/s12012-009-9059-x
- Wang CH, Jeng JS, Yip PK, Chen CL, Hsu LI, Hsueh YM, Chiou HY, Wu MM, Chen CJ (2002) Biological gradient between long-term arsenic exposure and carotid atherosclerosis. *Circulation* 105 (15):1804-1809
- Wang SL, Li WF, Chen CJ, Huang YL, Chen JW, Chang KH, Tsai LY, Chou KM (2011) Hypertension incidence after tap-water implementation: a 13-year follow-up study in the arseniasis-endemic area of southwestern Taiwan. *Sci Total Environ* 409 (21):4528-4535. doi:S0048-9697(11)00815-1 [pii]
10.1016/j.scitotenv.2011.07.058
- Watanabe T, Hirano S (2012) Metabolism of arsenic and its toxicological relevance. *Arch Toxicol*. doi:10.1007/s00204-012-0904-5
- WHO-IARC (2012) Arsenic and inorganic arsenic compounds. IARC Monographs 100C
- Yuan Y, Marshall G, Ferreccio C, Steinmaus C, Selvin S, Liaw J, Bates MN, Smith AH (2007) Acute myocardial infarction mortality in comparison with lung and bladder cancer mortality in arsenic-exposed region II of Chile from 1950 to 2000. *Am J Epidemiol* 166 (12):1381-1391. doi:kwm238 [pii]
10.1093/aje/kwm238
- Zhang C, Mao G, He S, Yang Z, Yang W, Zhang X, Qiu W, Ta N, Cao L, Yang H, Guo X (2012a) Relationship between long-term exposure to low-level arsenic in drinking water and the prevalence of abnormal blood pressure. *J Hazard Mater*. doi:S0304-3894(12)00969-7 [pii]
10.1016/j.jhazmat.2012.09.045
- Zhang W, Xue J, Ge M, Yu M, Liu L, Zhang Z (2012b) Resveratrol attenuates hepatotoxicity of rats exposed to arsenic trioxide. *Food Chem Toxicol* 51C:87-92. doi:S0278-6915(12)00696-5 [pii]
10.1016/j.fct.2012.09.023
- Zhao X, Feng T, Chen H, Shan H, Zhang Y, Lu Y, Yang B (2008a) Arsenic trioxide-induced apoptosis in H9c2 cardiomyocytes: implications in cardiotoxicity. *Basic Clin Pharmacol Toxicol* 102 (5):419-425. doi:PTO150 [pii]
10.1111/j.1742-7843.2007.00150.x

Zhao XY, Li GY, Liu Y, Chai LM, Chen JX, Zhang Y, Du ZM, Lu YJ, Yang BF (2008b) Resveratrol protects against arsenic trioxide-induced cardiotoxicity in vitro and in vivo. *Br J Pharmacol* 154 (1):105-113. doi:bjp200881 [pii]

10.1038/bjp.2008.81

Zierold KM, Knobeloch L, Anderson H (2004) Prevalence of chronic diseases in adults exposed to arsenic-contaminated drinking water. *Am J Public Health* 94 (11):1936-1937. doi:94/11/1936 [pii]